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An intervention trial to inhibit the progression of precancerous gastric lesions: compliance, serum micronutrients and *S-allyl* cysteine levels, and toxicity

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Gastric cancer is the second most frequent cause of death from cancer in the world and the leading cause of death from cancer in China. In September 1995, we launched a randomized multi-intervention trial to inhibit the progression of precancerous gastric lesions in Linqu County, Shandong Province, an area of China with one of the world's highest rates of gastric cancer. Treatment compliance was measured by pill counts and quarterly serum concentrations of vitamin C, vitamin E and *S-allyl* cysteine. In 1999, toxicity information was collected from each trial participant to evaluate treatment-related side-effects during the trial. Compliance rates were 93% and 92.9% for 39 months of treatment with the vitamins/mineral and garlic preparation, respectively. The means for serum concentrations of vitamins C and E were 7.2 μg/ml and 1695 μg/dl among subjects in the active treatment groups compared with 3.1 μg/ml and 752 μg/dl among subjects in the placebo treatment group, respectively. No significant differences in side-effects were observed between the placebo treatment group and the vitamins/mineral and garlic preparation treatment groups during the 39-month trial period.

Key words: Intervention Trial, Precancerous Gastric lesions, compliance, toxicity.

Introduction

Although gastric cancer mortality is declining in most industrialized countries, it is the second most frequent cause of death from cancer worldwide and the leading cause of cancer death in China (Pisani *et al.*, 1993; Li *et al.*, 1997). In 1995, we launched a 42-month randomized multi-intervention trial to inhibit the progression of precancerous gastric lesions in Linqu County, Shandong Province, an area of China with one of the world's highest rates of gastric cancer (70 per 100 000 men and 25 per 100 000 women per year). The design of this trial, compliance rates and acute side-effects during the initial phase, a 2-week treatment with amoxicillin 1 g b.i.d. and omeprazole 20 mg b.i.d. or placebo, were reported previously (Gail *et al.*, 1998). Herein we report data on compliance rates, reported symptoms, serum micronutrient and *S-allyl* cysteine (SAC) levels, and mortality during the subsequent 39-month period of daily nutritional intervention with vitamins/mineral and garlic preparation or placebo. A report on the progression of precancerous gastric lesions from September 1995 to February 1999 will be prepared following the completion of histopathological analysis.

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Materials and methods

The design and the initial phase of this trial are described in detail elsewhere (Gail *et al*., 1998). In brief, 3599 participants aged 35–69 years old were recruited from 13 villages selected at random within four townships of Linqu County, Shandong Province, China in September 1995. The study was approved by the Institutional Review Boards of the National Cancer Institute (NCI) and the Beijing Institute for Cancer Research (BICR), and all participants gave written informed consent. Three thousand four hundred and eleven subjects consented to participate in a randomized, double-blinded, 2^3-factorial intervention trial. Treatment with amoxicillin and omeprazole took place from September to November 1995 in the initial treatment phase (Gail *et al*., 1998) among 1108 subjects who had serologic evidence of *Helicobacter pylori* infection at baseline. In the second phase of the trial beginning in December 1995, all participants received either daily supplementation with 800 mg of garlic extract plus 4 mg steam-distilled garlic oil or placebo and/or with a mixture of 500 mg vitamin C, 200 IU vitamin E, 15 mg β-carotene and 75 μg selenium or placebo in a factorial design (Gail *et al*., 1998). The look-alike placebo capsules contained cellulose, lactose, and magnesium stearate. In June 1996, the β-carotene component of the vitamins/mineral supplement was discontinued (Gail *et al*., 1998).

Compliance measured by pill count

Each month, BICR field staff delivered boxes containing labelled pill bottles to trained volunteers in each village for distribution to the trial participants. If participants had any questions about the treatment schedule, local contacts were immediately available to provide the answers. During the period of vitamins/mineral and garlic preparation administration, field staff visited each participant once a month during the first 10 months and twice a month during the following 29 months to answer treatment-related questions and to promote pill compliance. If a subject was not home during the staff visit, an evening visit was scheduled. To help ensure compliance, 4th- and 5th-grade (11–12 years old) school children were recruited to visit trial participants on a daily basis on their way home from school.

Staff counted and recorded the number of pills remaining in each bottle before the new pill bottles were distributed each month. A subject was considered to be compliant for both vitamins/mineral and garlic preparation for a given month if all pill bottles were empty at the end of that month. If a subject was out of town or unlocatable at the time of pill counts, he or she was recorded as non-compliant.

Compliance measured by serum micronutrients

To ensure an adequate sample size, the design of the compliance study was to collect 5-ml blood samples from 64 randomly selected subjects from four villages in each quarter during the 39-month nutritional intervention phase. Within each village and within each of four treatment combinations (vitamins/mineral, active and placebo; garlic preparation, active and placebo), serum samples were collected from four randomly selected subjects. To allow for non-participation among the subjects, a total of five subjects per treatment combination were randomly selected, for a total of 80 potential samples. The fasting blood was obtained in the morning during unscheduled visits and allowed to clot in the dark at room temperature for 30–40 min.

After clotting, the blood was centrifuged at 1000 $\times g$ for 15 min. The resulting serum was aliquoted into three vials, stored immediately at −20°C, and then stored at −70°C within 2 or 3 days. Immediately after aliquoting, meta-phosphoric acid solution (6%) was added to the appropriate vials in preparation for the vitamin C assay. The analysis of vitamins C, E, and β-carotene was carried out by one of us (CSY). Vitamin C concentrations were determined according to the HPLC method of Zhang *et al*. (1990). An HPLC procedure (Chen *et al*., 2000) based on a previously described method (Miller and Yang, 1985; Sowell *et al*., 1994) was used for the determination of vitamin E, β-carotene, and other fat-soluble nutrients on duplicate 150 μl serum samples. In addition, serum concentrations of retinol, lutein and β-cryptoxanthin were measured as reference micronutrients that are not included in the interventions. β-Carotene samples also served as controls because only samples after June 1996, when β-carotene was discontinued, were used for β-carotene analyses. Concentration of serum SAC was assayed at the Wakunaga Company's laboratory in Japan (Imai *et al*., 1994). For each of these assays, laboratory staff at Rutgers University and Wakunaga were blinded to the treatment status of subjects providing samples.

Side-effects and mortality assessment

To assess whether any long-term treatment-related

side-effects occurred during phase 1 or 2 of the intervention trial since 1995, each trial participant was asked in February 1999 to respond to a comprehensive list of 53 questions. Respondents were read the list and asked to reply 'yes' or 'no' as to whether they had experienced a sign of symptom since the start of the trial. Mortality data, including sex, age of death, cause of death and diagnostic information, were collected in each quarter during the trial.

Statistical analysis

Monthly population-based compliance rates for vitamins/mineral and garlic preparation pills were calculated as the number of subjects with no pills in each bottle that month divided by the number of subjects living at the end of the month. The overall compliance rates for the 39-month period were calculated as the mean of monthly compliance rates for the entire study population. Means and standard deviations for vitamins C, E and lutein/zeaxanthin were calculated. Geometric means were calculated by exponentiating the means of the logarithms of quarterly serum concentrations of β-carotene, β-cryptoxanthin and SAC. A two-sided t-test was used to assess whether the means of overall serum concentrations differed between intervention and placebo groups for vitamins C, E, SAC, retinol and lutein/zeaxanthin. Similarly, a two-sided t-test was performed on the means of logarithms of SAC and β-cryptoxanthin. We treated these measurements as independent in trial analyses even though a few subjects were selected at random more than once. Side-effects, overall mortality and cardiovascular or cancer deaths were tabulated by intervention type (garlic preparation and vitamins/mineral) for the active and placebo treatment groups and evaluated using the chi-square test.

Results

As of April 1999, 3282 subjects (96.2%) were active participants among 3411 participants randomized at the start of the trial. A total of 129 subjects withdrew from the trial (deceased 81, refused 48) during the 39-month trial period. The overall average compliance rates were 93% and 92.9% for 39 months of treatment with vitamins/mineral and garlic preparation, respectively. On a month-by-month basis, the average overall compliance rate ranged from a low of 87% in month 29 to a high of 96% in months 14 and 26. Individual villages exhibited monthly compliance rates as low as 78% and as high as 98% (Figure 1).

Blood samples of 562 subjects from quarters 1–3 and 5–9 were collected to determine serum vitamin concentrations. Among these assayed subjects, 21.4% were selected at random twice and 4.7% were selected at random more than twice. Because less than 5 ml of blood was collected from a number of subjects, data on vitamin C data were available for 559 subjects and vitamin E for 545. Blood samples from 296 subjects for quarters 1–3 and 5–7 were assayed for SAC. The means presented in Table 1 are the mean concentrations of selected micronutrients and

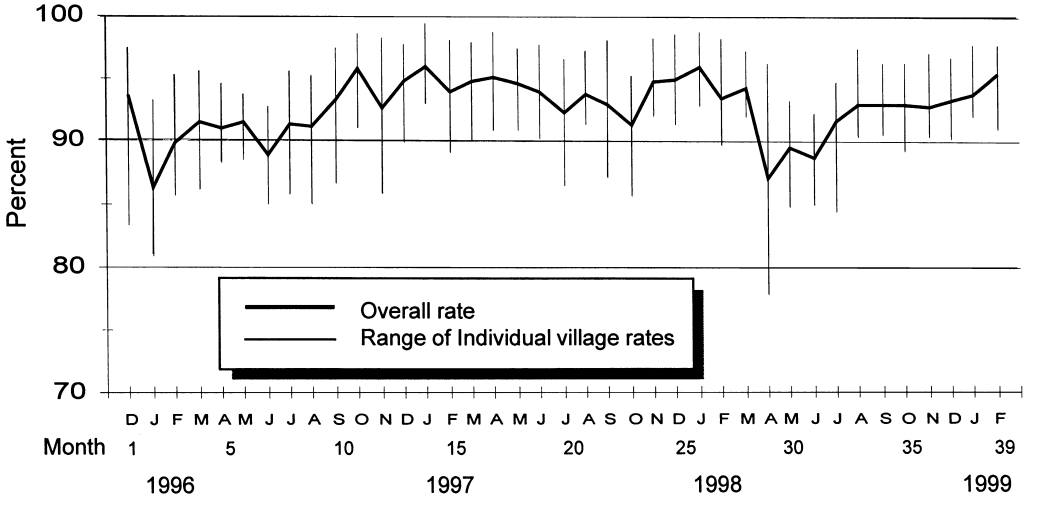

Figure 1. Percentage with 100% consumption of vitamin/garlic pills.

Table 1. Geometric[a] or arithmetic means and standard deviations (SD) for micronutrients and *S-allyl* cysteine concentrations in blood samples by treatment group for selected quarters[b]

Micronutrients	Active treatment group			Corresponding placebo group			*t*-test[c] *P*-value
	N	Mean	SD	*N*	Mean	SD	
Supplemented							
Vitamin C (μg/ml)	274	7.19	3.02	285	3.04	2.16	< 0.0001
Vitamin E (μg/dl)	277	1695.66	554.56	268	751.96	221.35	< 0.0001
S-allyl cysteine	147	57.53		149	35.08		
(ng/ml)[a]		(4.05)	(1.02)		(3.56)	(1.34)	0.0005
Not supplemented							
Retinol (μg/dl)	277	55.24	15.75	268	53.61	15.95	0.23
Lutein/zeaxanthin (μg/dl)	277	68.56	31.21	268	68.19	34.67	0.89
β-Cryptoxanthin	277	4.32		268	4.54		
(μg/dl)[a]		(1.46)	(0.81)		(1.51)	(0.84)	0.48

[a]The geometric mean is presented. The mean and standard deviation of $\log_e$ values are shown in parentheses.
[b]Quarters 1–3 and 5–9 for micronutrients; quarters 1, 3, and 5–7 for *S-allyl* cysteine.
[c]*t*-test was performed in the original values of vitamins C, E, retinol and lutein/zeaxanthin and on the natural logarithms of *S-allyl* cysteine and β-cryptoxanthin.

SAC. The means of vitamins C and E were 7.2 μg/ml and 1695 μg/dl among subjects in the active treatment group compared with 3.0 μg/ml and 752 μg/dl among subjects in the placebo treatment group, respectively ($P < 0.0001$). The serum means of retinol, lutein/zeaxanthin and β-cryptoxanthin, which were not supplemented, were about the same in the active and placebo treatment groups. The serum concentration of SAC was significantly higher in the active treatment group (57.5 ng/ml) than in the placebo treatment group (35.1 ng/ml) ($P = 0.0005$).

The mean serum concentrations of vitamin E were consistently and significantly higher in the active treatment group than in the placebo treatment group in each quarter (Figure 2). A similar difference in the serum concentrations of vitamin C was observed between the active treatment group and the placebo treatment group in each quarter (data not shown). The mean serum β-carotene concentration was significantly higher in the active treatment group (53.4 μg/dl) than the placebo group (34.9 μg/dl) in quarter 1, after a 3-month supplementation with 15 mg daily β-carotene, but no differences in the serum concentrations of β-carotene were observed between the active and placebo groups after the β-carotene was dropped from the vitamins/mineral intervention in June 1996 (Figure 3).

Among trial participants interviewed for side-effects in February 1999, 944 (49.2%) subjects given garlic or vitamins/mineral supplement reported

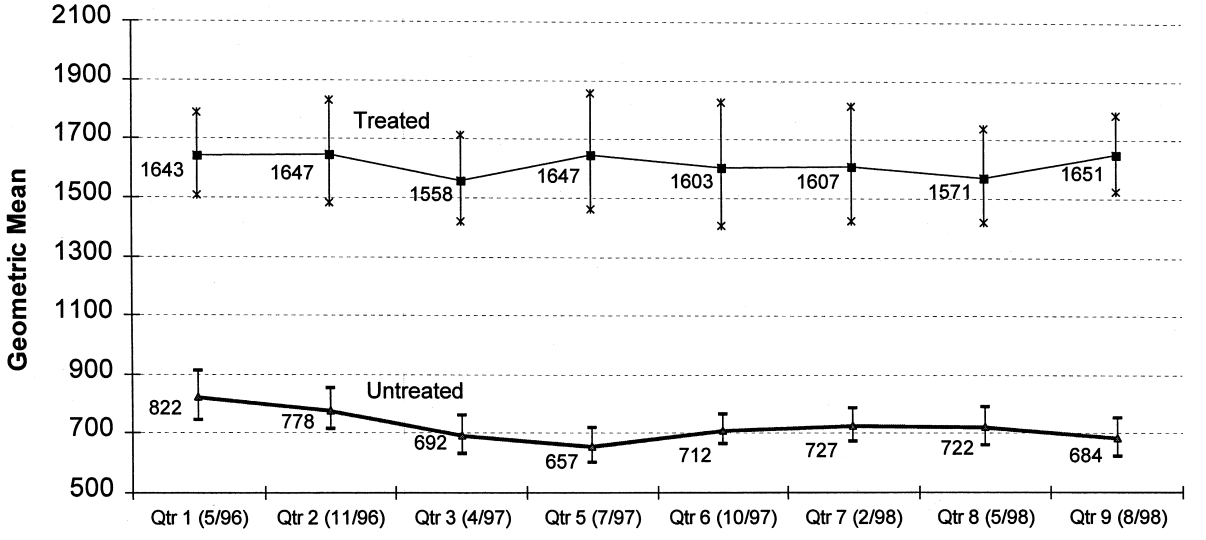

Figure 2. Geometric means and 95% confidence intervals of α-tocopherol (μg/dl) for treated and placebo groups.

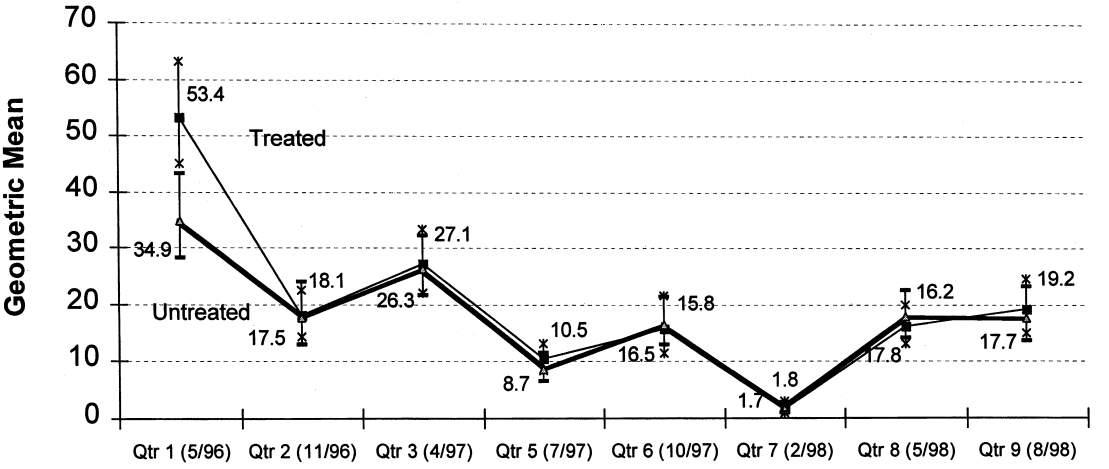

Figure 3. Geometric means and 95% confidence intervals of β-carotene (μg/dl) for treated and placebo groups.

having at least one side-effect, compared with 976 (50.8%) subjects in the placebo groups ($P > 0.05$). Overall, heartburn (garlic supplement 14.1% versus placebo 15.1% and vitamins/mineral supplement 14.7% versus placebo 14.6%), oral and gastric irritation (garlic supplement 18.0% versus placebo 19.6% and vitamins/mineral supplement 18.2% versus placebo 19.4%), loss of appetite (garlic supplement 26.7% versus placebo 29.3% and vitamins/mineral supplement 27.2% versus placebo 28.9%), and common cold (garlic supplement 18.3% versus placebo 18.7% and vitamins/mineral supplement 17.7% versus placebo 19.3%) were the most common symptoms reported among the trial participants, but no significant differences were observed between garlic preparation and vitamins/mineral treatment groups and their corresponding placebo groups or any other side-effects. Of the 3411 trial participants, a total of 81 were reported to have died after the trial began. There was no association between any of the treatments and death from all causes, cardiovascular diseases, total cancer or gastric cancer (Table 2).

Discussion

This report describes a high overall compliance rate for treatment with vitamins/mineral and garlic preparation during a 39-month period of nutritional intervention in Linqu County, China, a high-incidence area for stomach cancer. Our compliance rate is consistent with the compliance rate reported in a recently completed vitamin intervention trial in Linxian, a rural area with high rates of esophageal cancer (Blot *et al*., 1993). In the initial *H. pylori* treatment phase of our trial, 98% of participants took every amoxicillin and omeprazole or placebo pill according to the protocol schedule (Gail *et al*., 1998). It is noteworthy that the lowest compliance rate in the current nutrition intervention occurred in January and February during the Chinese New Year,

Table 2. Number of deaths in each treatment or placebo group

	Active treatment group ($N = 1706$) No. of deaths	Corresponding placebo group ($N = 1705$) No. of deaths	χ^2 test P
Vitamins/mineral			
Total deaths	38	43	0.57
Cancer deaths	23	17	0.34
Gastric cancer deaths	6	4	0.53
Cardiovascular deaths	9	12	0.51
Garlic preparation			
Total deaths	47	34	0.14
Cancer deaths	24	16	0.20
Gastric cancer deaths	5	5	1.00
Cardiovascular deaths	12	9	0.51

suggesting that social activity in this rural area may influence the compliance rate.

Several important factors have contributed to the continued high compliance in this trial. BICR field investigators have worked in Linqu since 1983 and have developed close working relationships with villagers, community leaders and local health authorities. The trial has received continued strong support from both village leaders and Linqu health officials. Successful gastric cancer screening programmes conducted previously in this area by BICR and NCI helped enhance villager trust. Other important factors included high levels of self-motivation on the part of trial participants, a stable population with limited loss to follow-up, and effective management of trial activities by study staff. Management strategies included twice-monthly visits by field staff to conduct interim pill counts, answer questions, and encourage the continued interest and cooperation of trial participants, and visits by village school children to remind participants to take all their pills. Field staff also monitored each village's compliance rate and acted immediately to encourage pill taking in villages where compliance had fallen. Despite the limitations of the use of pill counts to measure compliance (Friedman *et al*., 1981; Rudd *et al*., 1989a,b), it is reassuring that the high estimates of compliance based on pill counts in this trial are supported by clear differences in serum micronutrients and SAC between active and placebo groups.

Levels of vitamin C (short metabolic half-life), vitamin E (long metabolic half-life) and SAC based on serum sample measurements, were significantly higher among subjects in the active treatment groups compared with the placebo group. Mean serum levels of vitamins C and E in the placebo group remained consistently low throughout the trial period and were similar to levels in Linqu in early 1990 (Zhang *et al*., 1994), and to levels among the general population in Linxian (Ershow *et al*., 1984; Yang *et al*., 1985). Among participants in the active treatment groups, serum levels increased more than 100% for vitamins C and E, and 60% for SAC, after supplementation with vitamins/mineral and garlic preparation, respectively. The mean serum vitamin C level in the active treatment group was close to that reported for the US population in 1976–1980 (US Department of Health and Human Services, 1982). Serum levels of β-cryptoxanthin, lutein/zeaxanthin and retinol, which served as internal controls, were not increased in the active treatment arms. Laboratory monitoring of intervention regimens provides more objective evidence for subject compliance than pill counts, as well as quantification of serum levels of the agents used for intervention. Because neither trial subjects nor the field staff knew the schedule of blood collection in advance and the trial subjects fasted in the early morning without taking any pills on the day of blood draw, the measured serum micronutrient concentrations were likely to reflect the true levels in the blood.

The percentage of side-effects reported was similar in the active and placebo-treated subjects, suggesting that the use of vitamins/mineral and garlic preparation supplements in the trial was safe.

In conclusion, our study demonstrated that daily long-term use of 500 mg vitamin C, 200 IU vitamin E, 75 μg selenium and 800 mg garlic preparation + 4 mg garlic oil was well tolerated and was associated with no more side-effects than placebo treatment. The lack of treatment-related side-effects, in addition to effective trial management and high motivation of study participants, may have contributed to the high compliance rates achieved in this trial.

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